Response to: Depolarization vs. repolarization: what is the mechanism of ventricular arrhythmogenesis underlying sodium channel haploinsufficiency in mouse hearts?

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We thank the authors of this letter for their positive comments on our work and contributions. We point out that their comments in fact do not bear directly on the content of our review. The latter concerns effects of conduction change rather than recovery from excitation on arrhythmic behaviour. Their comments concern readouts from the action potential recovery that follows. In this the authors express strong reservations concerning the use of monophasic action potential recordings. Our own view corresponds more closely to accepted and long established criteria for such measurements accurately reproducing waveform morphology and repolarization times of transmembrane APs (Knollmann et al., 2001) potentially translatable from studies in mouse to human hearts (Moore and Franz, 2007), that precede the account given by the correspondent (Tse et al., 2016c). All techniques possess limitations: however, available readouts of APD recovery under common conditions in fact show a close consistency in the papers quoted by the correspondent (Martin et al., 2010, Stokoe et al., 2007). This is evident in absolute values of APD_{70} in the left ventricles of WT heart paced at 8 Hz, summarized in the bar graphs of their Figure 9A and C (Stokoe et al., 2007) and Figure 4B (Martin et al., 2010) respectively. The first paper then achieves a statistical power to demonstrate significant changes with pharmacological challenge in the LV specifically. It did not explore other cardiac regions within the same preparation, but went on to segment the test, *Scn5a*$^{+/-}$, hearts into subcategories by their different arrhythmic phenotypes. The second continues from the first and studied all LV and RV, endocardial and epicardial, regions often in the same heart, confirming concordant directions of change in all regions studied (Their figure 3A-F), and statistical significance in the RV epicardium, where such effects would therefore be greatest (Their figure 4B). Furthermore,

(Stokoe et al., 2007) sorted *Scn5a*$^{+/-}$ hearts by arrhythmic property for detailed analysis relating these properties to refractory period. In contrast, (Martin et al., 2010) differently treated the *Scn5a*$^{+/-}$ group of hearts together. Both papers meticulously reported all remaining APD_x values, but the inferences concerning relative AP recovery timecourses in *Scn5a*$^{+/-}$ and WT, before or after flecainide or quinidine challenge, particularly changes in the right ventricle epicardium relative to remaining regions remained the same.

The correspondent does not seem to have noted our detailed description, controls and caveats in our Methods concerning electrode distances when measuring activation latencies. We draw his attention to p 1153, column 2 of the paper (Martin et al., 2010): 'The stimulating electrode and the epicardial LV and RV recording electrodes were clamped at a constant position through all experiments. This was at a distance of approximately 10 mm between stimulating and each recording electrode, although it was slightly larger for the LV than for the RV. This distance allowed the hearts to be placed into the rig, with the stimulating electrode coming into contact with the septum and the recording electrodes coming into contact with the left and right ventricles. While the direct absolute distance between electrodes would not necessarily reflect the path through which the electrical signal would be conducted in the spherical whole heart, the fact that the distance was maintained between experiments allowed consistent measurements of activation latencies. As the clamp was secure throughout experiments, this distance was constant to ~0.5 mm, i.e., 5% of the distance between stimulating and recording electrodes.' and ' Activation latencies for MAPs recorded from the RV and LV epicardium were measured from the stimulus time to peak amplitude of the MAP to give an indication of conduction velocities. With the positions of the stimulating and recording electrodes constant between experiments, comparisons could be made between WT and *Scn5a*$^{+/-}$ hearts, and before and after drug. However, as the distance from stimulating electrode on the septum was slightly greater to the LV recording electrode than the RV recording electrode, no direct comparison could be made between LV and RV.'(Martin et al., 2010).

We agree with the correspondent's view concerning the potential importance of propagation parameters as reflected in our experimental analysis concerning AP wavelength (Matthews et al., 2013), but do not regard this as a sole determinant of arrhythmic tendency. Thus: (1) within the *Scn5a*$^{+/-}$ platform under discussion, Martin et al. (2010) had pointed out that: '….*Scn5a*$^{+/-}$ hearts showed slowed conduction times in both RV and LV, exacerbated not only by flecainide but also by quinidine, in contrast to their differing effects on arrhythmogenesis.' (2) We point out evidence for contributions from recovery, APD and refractoriness parameters in the *Scn5a*+/ΔKPQ system modeling long QT as opposed to Brugada Syndrome in which the consequent increase in wavelength accompanied increased, and not decreased arrhythmic tendency (Sabir et al., 2007b, Sabir et al., 2007a). (3) We point out situations of altered Ca^{2+} homeostasis in which arrhythmic tendency appears to accompany normal conduction and action potential recovery (Hothi et al., 2008). (4) We point out that (Tse et al., 2016a) themselves report arrhythmic effects of hypokalaemia in an absence of altered conduction more consistent with (2) above rather than their own assertion related to conduction velocity.

However, we then are unclear as to the grounds that the correspondents bases their assertion as this is based on quoted papers themselves solely dependent upon measures of recovery processes obtained from the very MAP electrode measurements, about which he

expresses significant doubt. Similarly their quoted papers on analysis of restitution properties (Tse et al., 2016b, Tse et al., 2016a) were not based on the analysis of the kind we have previously described that tested for a central role of wavelength change in arrhythmia (Matthews et al., 2013). Rather they were based on analysis of MAP electrode-derived recovery (APD) data (Tse et al., 2016c, Tse et al., 2016d). Information about these processes would be required in order to reconstitute the wavelength term.

We also agree with their suggestions concerning the usefulness of studies using complementary techniques, and draw attention to available multi-electrode recording studies on *Scn5a*$^{+/-}$ (Martin et al., 2011, Zhang et al., 2014, Jeevaratnam et al., 2012, Jeevaratnam et al., 2011) and other systems (Salvage et al., 2015). However these provide measures of *propagation*, and not *recovery* and in that respect do not 'supplement findings from MAPs' (Martin et al., 2011). We also draw their attention to complementary transmembrane membrane potential studies we have indeed performed on the same systems (King et al., 2013). We look forward to corresponding reports on their own experimental platform in the near future.

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